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## ORIGINAL ARTICLE

# Iron deficiency, anemia, and mortality in renal transplant recipients

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## SUMMARY

Anemia, iron deficiency anemia (IDA), and iron deficiency (ID) are highly prevalent in renal transplant recipients (RTR). Anemia is associated with poor outcome, but the role of ID is unknown. Therefore, we aimed to investigate the association of ID, irrespective of anemia, with all-cause mortality in RTR. Cox regression analyses were used to investigate prospective associations. In 700 RTR, prevalences of anemia, IDA, and ID were 34%, 13%, and 30%, respectively. During follow-up for 3.1 (2.7–3.9) years, 81 (12%) RTR died. In univariable analysis, anemia [HR, 1.72 (95% CI: 1.11–2.66),  $P = 0.02$ ], IDA [2.44 (1.48–4.01),  $P < 0.001$ ], and ID [2.04 (1.31–3.16),  $P = 0.001$ ] were all associated with all-cause mortality. In multivariable analysis, the association of anemia with mortality became weaker after adjustment for ID [1.52 (0.97–2.39),  $P = 0.07$ ] and disappeared after adjustment for proteinuria and eGFR [1.09 (0.67–1.78),  $P = 0.73$ ]. The association of IDA with mortality attenuated after adjustment for potential confounders. In contrast, the association of ID with mortality remained independent of potential confounders, including anemia [1.77 (1.13–2.78),  $P = 0.01$ ]. In conclusion, ID is highly prevalent among RTR and is associated with an increased risk of mortality, independent of anemia. As ID is a modifiable factor, correction of ID could be a target to improve survival.

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## Key words

anemia, iron deficiency, kidney transplantation, mortality

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## Introduction

Post-transplant anemia is associated with an increased risk of graft failure, cardiovascular mortality, and all-cause mortality in renal transplant recipients (RTR) [1–3]. Iron deficiency (ID) is highly prevalent in RTR and is one of the main contributors to post-transplant anemia [4,5]. The decreased intestinal uptake of iron as a consequence of increased hepcidin and IL-6 concentrations, which exist as a result of the pro-inflammatory state

that renal transplantation constitutes [6,7], may contribute to a frequent occurrence of functional ID. In addition, increased consumption of iron as a consequence of enhancement of erythropoiesis after successful transplantation in response to recovery of renal function may further augment the functional ID [8]. Inadequate iron stores at the time of transplantation, blood loss during the surgical procedure, and frequent post-transplant venipunctures may also contribute to the occurrence of ID [8]. It has indeed been shown that 60% of

RTR without ID at the time of transplantation developed ID in a period of 6 months after transplantation [9].

Conventionally, ID is linked to anemia. However, in addition to its role in hemoglobin and oxygen transport, iron plays a pivotal role in enzyme activity of a number of enzymes linked to energy metabolism and in other oxygen-binding proteins such as myoglobin [10]. To date, potential consequences of ID (with and without anemia) in transplantation are unknown. The aim of this study was to validate the impact of anemia, and to assess the impact of iron deficiency anemia (IDA) and ID prospectively on all-cause mortality in RTR.

## Methods

### Study population

All RTR (aged  $\geq 18$  years) that were at least 1 year post-transplantation were approached for participation during outpatient clinic visits between 2008 and 2011, as described previously [11]. RTR were all transplanted at the University Medical Center Groningen, Groningen, the Netherlands, and had no history of drug or alcohol abuse, as reported in the patient records. Written informed consent was obtained from 707 (87%) from the 817 initially invited RTR. For the analyses, we excluded patients with missing data on iron status parameters ( $n = 7$ ), resulting in 700 RTR eligible for analyses. The study protocol was approved by the institutional review board (METc 2008/186). The study protocol adhered to principles of the Declaration of Helsinki and was consistent with the Principles of the Declaration of Istanbul as outlined in the 'Declaration of Istanbul on Organ Trafficking and Transplant Tourism'.

### Iron status parameters

Blood was drawn in the morning. Transferrin was measured using an immunoturbidimetric assay (Cobas c analyzer, Modular P system, Roche diagnostics, Mannheim, Germany). Serum ferritin concentrations were determined using the electrochemiluminescence immunoassay (Modular analytics E170, Roche diagnostics, Mannheim, Germany). Serum iron was measured using photometry (Modular P800 system; Roche diagnostics, Mannheim, Germany). Transferrin saturation [TSAT (%)] was calculated as  $100 \times \text{serum iron } (\mu\text{mol/l}) / 25 \times \text{transferrin (g/l)}$  [12]. ID was defined as

TSAT  $< 20\%$  and ferritin  $< 300 \mu\text{g/l}$ . Anemia was defined as Hb  $< 13 \text{ g/dl}$  (M) or  $< 12 \text{ g/dl}$  (F).

### Statistical analysis

Data were analyzed using IBM SPSS software, version 22.0 (SPSS Inc., Chicago, IL, USA) and R version 3.0.1 (Vienna, Austria). Data were expressed as mean  $\pm$  SD when normally distributed or as median with interquartile range (IQR) in the case of skewed distribution. The baseline characteristics of patients without anemia and ID, and patients with anemia, IDA, or ID are shown in Table 1.

Kaplan–Meier curves were used to demonstrate the effect of the presence of ID and/or anemia, anemia, IDA, and ID on survival. Differences in survival rates were tested using the Cox–Mantel log-rank test.

Cox regression analyses were used to investigate prospective associations of anemia, IDA, and ID with all-cause mortality. Various models were built to adjust for potential confounders. Model 1 was considered as crude Cox regression analysis. Model 2 was adjusted for age and sex; model 3 was additionally adjusted for anemia in the case of ID or for ID in the case of anemia; model 4 was additionally adjusted for eGFR and proteinuria.

As secondary analyses for the association between anemia, IDA, and ID with mortality, we adjusted for several potential confounders in multivariable Cox regression models (Table 3). After adjustment for age, sex, eGFR, and proteinuria, we adjusted in separate models for lifestyle factors and comorbidities (model 2; diabetes mellitus, systolic blood pressure, BMI, alcohol use, and smoking), for medication use (model 3; ACE inhibitors, diuretics, and CNI inhibitors), for inflammation (model 4; hs-CRP), and for heart failure (model 5; NT-proBNP).

As sensitivity analysis, we assessed the association of hemoglobin as continuous variable with mortality in the multivariable analysis rather than anemia as a dichotomous variable and by using another definition of ID, namely TSAT  $< 20\%$  and ferritin  $< 200 \mu\text{g/l}$  [13]. Regarding the specific iron status parameters, we assessed the association between serum ferritin, TSAT, and serum iron on all-cause mortality in univariable and multivariable Cox regression analyses. We used Cox regression analyses with restricted cubic splines with three knots to test for potential nonlinearity of the prospective associations of ln-transformed ferritin, TSAT, and serum iron with all-cause mortality. All tests were two-sided, and a  $P$ -value of  $< 0.05$  was considered statistically significant.

**Table 1.** Baseline characteristics.

Variables	Patients without anemia and without ID (n = 344)	Patients with anemia (n = 237)	Patients with IDA (n = 90)	Patients with ID (n = 208)
<i>Demographics</i>				
Age (years)	53 ± 12	53 ± 14	55 ± 13	54 ± 12
Sex (male, %)	132 (38)	131 (55)	46 (51)	99 (48)
BMI (kg/m <sup>2</sup> )	27 ± 5	26 ± 5	27 ± 5	28 ± 5
Systolic blood pressure (mmHg)	135 ± 16	138 ± 19	140 ± 18	137 ± 17
Diastolic blood pressure (mmHg)	82 ± 10	82 ± 12	82 ± 11	83 ± 11
Never smoker (%)	41	38	36	36
Former smoker (%)	38	46	56	50
Current smoker (%)	13	11	7	9
Unknown smoking status (%)	8	4	2	5
No alcohol consumption (%)	9	10	12	9
Alcohol 0–10 (g/day)	52	59	64	68
Alcohol 10–30 (g/day)	22	19	18	15
Alcohol >30 (g/day)	6	3	0	2
<i>Renal parameters</i>				
Time since transplantation (years)	6.4 (3.0–12.8)	4.7 (1.2–11.2)	3.7 (1.0–9.3)	4.3 (1.1–10.0)
eGFR (ml/min/1.73 m <sup>2</sup> )	58.5 ± 19.2	42.1 ± 17.8	44.6 ± 18.6	50.4 ± 19.6
Proteinuria (≥0.5 g/24 h) (%)	16	33	34	28
<i>Comorbidities</i>				
Anemia (%)	0	100	100	43
Diabetes mellitus (%)	21	24	37	35
<i>Treatment</i>				
ACE inhibitor (%)	31	39	30	26
Angiotensin II antagonist (%)	13	18	20	18
Beta-blocker (%)	62	65	67	66
Diuretic (%)	35	48	56	49
Calcineurin inhibitor (%)	49	68	72	66
Proliferation inhibitor (%)	82	84	84	86
Statin use (%)	54	55	54	49
mTOR inhibitor (%)	2	2	4	2
EPO-stimulating agents (%)	2	4	4	2
Iron supplements (%)	4	9	6	5
<i>Laboratory measurements</i>				
Hb (g/dl)	14.3 ± 1.1	11.4 ± 1.0	11.1 ± 1.0	12.7 ± 1.8
MCV (fl)	91 ± 5	90 ± 7	87 ± 7	88 ± 6
Iron (μmol/l)	18 ± 5	13 ± 6	8 ± 3	9 ± 3
Ferritin (μg/l)	156 (86–257)	97 (43–203)	37 (21–69)	46 (27–97)
TSAT (%)	31 ± 9	23 ± 12	12 ± 5	14 ± 4
NT-pro-BNP (pg/ml)	159 (72–393)	425 (197–1090)	550 (245–2299)	350 (127–1069)
hs-CRP (mg/l)	1.3 (0.6–3.5)	1.7 (0.8–4.9)	2.9 (0.8–6.5)	2.5 (1.0–6.3)

Azathioprine and mycophenolate mofetil were considered as proliferation inhibitors; cyclosporine and tacrolimus as calcineurin inhibitors. Diabetes mellitus was defined as serum glucose >7 mmol/l or the use of antidiabetic drugs.

## Results

### Baseline characteristics

We included 700 RTR (age 53 ± 13 years; 57% males) with a median (interquartile range) duration after transplantation of 5.4 (1.9–12.0) years. Mean eGFR was 52.3 ± 20.2 ml/min/1.73 m<sup>2</sup>. Mean hemoglobin concentration was 13.2 ± 1.8 g/dl, serum iron

concentration was 15.3 ± 6.0 μmol/l, ferritin concentration was 118 (55–222) μg/l, and TSAT was 25 ± 11%. Anemia, iron deficiency anemia (IDA), and ID occurred in 237 RTR (34%), 90 RTR (13%), and 208 RTR (30%), respectively (Fig. 1). Mean corpuscular volume (MCV) was 90 ± 7 fl in the anemic RTR, 87 ± 7 fl in those with IDA, and 88 ± 6 fl in those with ID. RTR with anemia were more often male, had the lowest eGFR, and used more ACE inhibitors compared with

those with IDA and ID. RTR with IDA were at shorter duration after transplantation, had the highest systolic blood pressure, had higher concentrations of C-reactive protein and NT-proBNP levels, and had the highest prevalence of diabetes mellitus as comorbidity as compared to the other patients with anemia or with ID. In contrast, RTR with ID were more often female and had a higher eGFR compared with the RTR with anemia and IDA (Table 1).

### Anemia, IDA, ID, and mortality

During a median follow-up for 3.1 (2.7–3.9) years, 81 (12%) RTR died, of which 38 (47%) due to cardiovascular causes. Other causes of death were infection (24%), malignancy (16%), and miscellaneous and other causes (14%). Kaplan–Meier survival curves for RTR with or without ID and/or anemia, for RTR with and without anemia, for RTR with and without IDA, and for RTR with and without ID are shown in Fig. 2. It appears that there is a marked difference in survival between RTR having anemia, IDA, or ID compared with those without (log-rank test  $P = 0.01$  for anemia;  $P < 0.001$  for IDA, and  $P = 0.001$  for ID).

In univariable Cox regression analysis, anemia [HR, 1.72 (95% CI: 1.11–2.66),  $P = 0.02$ ], IDA [2.44 (1.48–4.01),  $P < 0.001$ ], and ID [2.04 (1.31–3.16),  $P = 0.001$ ] were associated with mortality (Table 2).

In multivariable Cox regression analysis models, the association of anemia with mortality remained significant after adjustment for age and sex [1.72 (1.11–2.66),  $P = 0.02$ ]. However, the association of anemia with mortality lost statistical significance after adjustment for ID [1.52 (0.97–2.39),  $P = 0.07$ ]. Moreover when additional adjustment was performed for eGFR and

proteinuria, the association of anemia with mortality disappeared altogether [1.09 (0.67–1.78),  $P = 0.73$ ].

The association of IDA with mortality remained after adjustment for age and sex [2.09 (1.27–3.45),  $P = 0.004$ ]. When additional adjustment was performed for eGFR and proteinuria, the association of IDA with mortality lost significance [1.67 (0.99–2.82),  $P = 0.05$ ].

The association of ID with mortality remained after adjustment for age and sex [1.94 (1.25–3.01),  $P = 0.003$ ]. Further adjustment for anemia did not materially affect the association of ID with mortality [1.77 (1.13–2.78),  $P = 0.01$ ]. When additional adjustment was performed for eGFR and proteinuria, the association of ID with mortality remained significant [1.74 (1.10–2.73),  $P = 0.02$ ; Table 2].

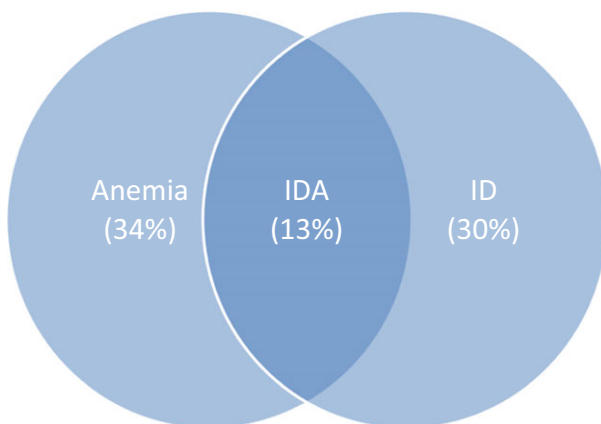
### Secondary analyses of association with mortality

In secondary analyses, we aimed to investigate whether the association of anemia, IDA, and ID with mortality is independent of other potential confounders (comorbidities, medication use, inflammation, and heart failure; Table 3). In these analyses, in which we adjusted for these other potential confounders, the associations of anemia and ID with mortality remained materially unchanged, that is, virtually absent for anemia and significant for ID, while that of IDA remained independent of adjustment for most of the potential confounders, but lost significance after adjustment for NT-proBNP (Table 3).

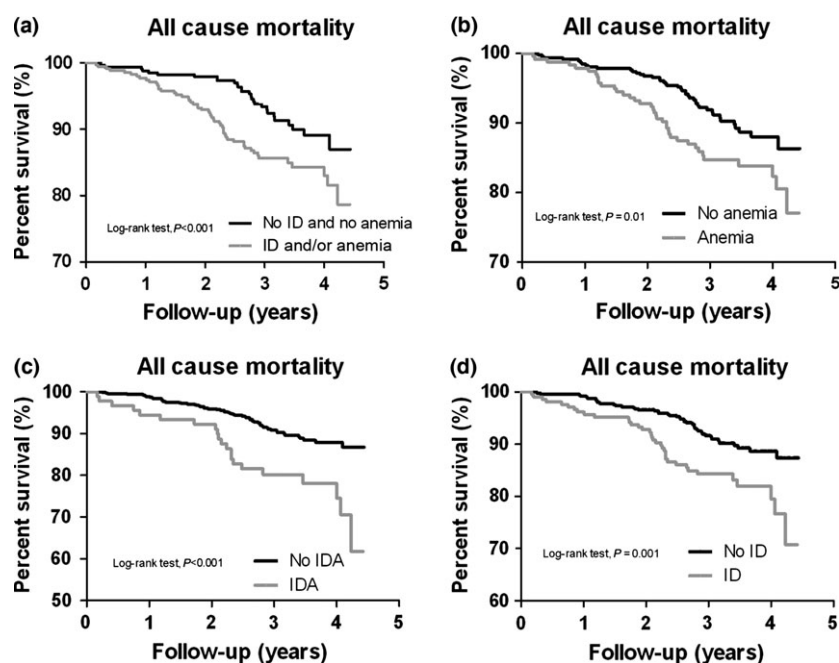
### Definition of ID and individual ID definition components

As sensitivity analysis, after adjustment for age, sex, eGFR, and proteinuria, we adjusted the association of ID with mortality for hemoglobin as continuous variable rather than anemia as a dichotomous variable. The association became weaker after adjustment for hemoglobin as continuous variable [1.54 (0.97–2.45),  $P = 0.07$ ]. In another sensitivity analysis, we assessed the association of ID with all-cause mortality using an alternative definition of ID (TSAT < 20% and ferritin < 200 µg/l). The association of this definition of ID with mortality remained also materially unchanged after adjustment for age, sex, eGFR, proteinuria, and anemia [1.66 (1.05–2.63),  $P = 0.03$ ].

Next to the definition of ID, we tested the individual iron status parameters, that is, serum ferritin, TSAT, and serum iron. In univariable analysis, In serum



**Figure 1** Prevalences of anemia, IDA, and ID in the RTR cohort.



**Figure 2** Kaplan–Meier curves for the difference in patient survival in (a) renal transplant recipients with or without ID and/or anemia (b) with or without anemia; (c) with or without iron deficiency anemia (IDA); (d) with or without iron deficiency (ID).

**Table 2.** Cox proportional hazard analysis for anemia, IDA, and ID in predicting all-cause mortality.

Variable	Anemia		IDA		ID	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Univariable	1.72 (1.11–2.66)	0.02	2.44 (1.48–4.01)	<0.001	2.04 (1.31–3.16)	0.001
Model 1	1.72 (1.11–2.66)	0.02	2.09 (1.27–3.45)	0.004	1.94 (1.25–3.01)	0.003
Model 2	1.52 (0.97–2.39)	0.07	—	—	1.77 (1.13–2.78)	0.01
Model 3	1.09 (0.67–1.78)	0.73	1.67 (0.99–2.82)	0.05	1.74 (1.10–2.73)	0.02

Model 1: Adjustment for age and sex.

Model 2: Model 1 + adjustment for ID (outcome: anemia) or anemia (outcome: ID).

Model 3: Model 2 + adjustment for eGFR and proteinuria.

ferritin as continuous variable was not associated with risk of mortality [1.01 (0.81–1.26),  $P = 0.95$ ]. This is likely the consequence of a nonlinear relationship of ferritin with mortality. In Fig. 3a, a cubic restricted spline depicting the U-shaped association of ferritin with all-cause mortality is shown. Indeed, we found significant deviances from linear associations of  $\ln$  ferritin with all-cause mortality ( $P = 0.03$ ), which lost statistical significance after adjustment for the potential confounders ( $P = 0.07$ ). When divided into quintiles, the lowest quintile [2.48 (1.08–5.79),  $P = 0.03$ ] and the highest quintile of ferritin concentrations [2.61 (1.16–5.87),  $P = 0.02$ ] were associated with higher risk of mortality when compared with the fourth quintile, in a model in

which we adjusted for potential confounders (age, sex, eGFR, proteinuria, and anemia). In further adjustment for hs-CRP, the strength of the relationship in the lowest quintile [2.22 (0.95–5.18),  $P = 0.07$ ] and in the highest quintile of ferritin [2.35 (1.04–5.29),  $P = 0.04$ ] decreased moderately.

In univariable analysis, TSAT as a continuous variable was inversely associated with risk of mortality [0.97 (0.95–0.99),  $P = 0.007$ ] (Fig. 3b). After adjustment for potential confounders (age, sex, eGFR, proteinuria, and anemia), a trend remains, but statistical significance is lost [0.98 (0.96–1.00),  $P = 0.06$ ].

Serum iron as a continuous variable was in univariable analysis inversely associated with risk of mortality



**Table 3.** Secondary analysis for the association of anemia, IDA, and ID with all-cause mortality.

Variable	Anemia		IDA		ID	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Model 1	1.19 (0.73–1.95)	0.48	1.67 (0.99–2.82)	0.05	1.76 (1.12–2.75)	0.01
Model 2	1.20 (0.74–1.96)	0.46	1.78 (1.04–3.05)	0.04	1.83 (1.16–2.89)	0.009
Model 3	1.29 (0.79–2.10)	0.31	1.86 (1.05–3.28)	0.03	1.75 (1.08–2.83)	0.02
Model 4	1.24 (0.76–2.01)	0.39	1.79 (1.03–3.11)	0.04	1.81 (1.13–2.90)	0.01
Model 5	1.22 (0.75–1.99)	0.42	1.74 (1.02–2.98)	0.04	1.76 (1.10–2.80)	0.02
Model 6	1.12 (0.68–1.85)	0.66	1.48 (0.83–2.65)	0.19	1.71 (1.06–2.76)	0.03

Model 1: Adjustment for age, sex, eGFR, and proteinuria.

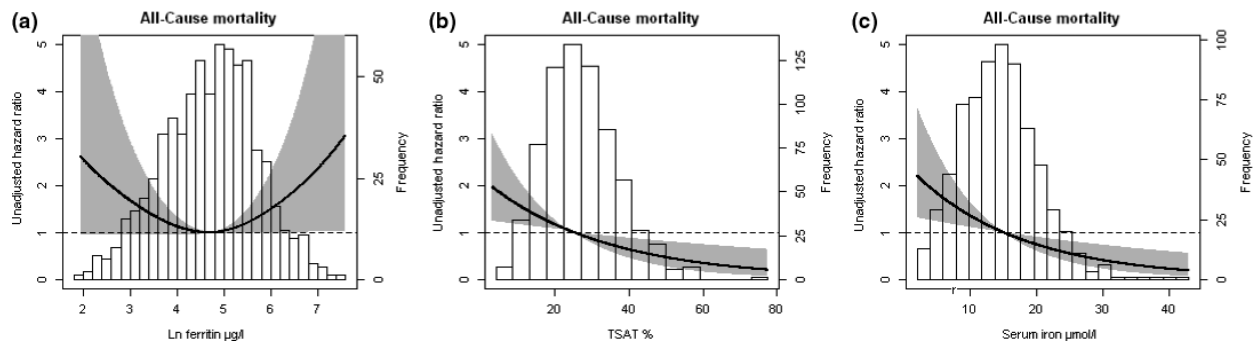
Model 2: Model 1 + adjustment for time since transplantation.

Model 3: Model 2 + adjustment for diabetes mellitus, SBP, BMI, alcohol, and smoking.

Model 4: Model 2 + adjustment for medication use (ACE inhibitors, diuretics, CNI inhibitors, and iron supplements).

Model 5: Model 2 + adjustment for inflammation (hs-CRP).

Model 6: Model 2 + adjustment for NT-pro-BNP.

**Figure 3** Associations between serum ferritin (a), TSAT (b), and serum iron (c) and risk of all-cause mortality. The line in the graph represents the risk of all-cause mortality. The gray area represents the 95% CI of the HR.

[0.94; (0.90–0.98),  $P = 0.003$ ] (Fig. 3c). After adjustment for potential confounders (age, sex, eGFR, proteinuria, and anemia), the association weakened [0.96 (0.92–1.00),  $P = 0.08$ ].

## Discussion

The main finding of this study was that ID, independently of anemia, is prospectively associated with all-cause mortality in stable RTR. We confirmed previous reports that anemia is associated with an increased risk of mortality in RTR [2,14]. However, in our study, the association of anemia with all-cause mortality lost statistical significance after adjustment for ID. The association was lost altogether after additional adjustment for renal function, whereas the association of ID with mortality was not influenced by either anemia or renal

function. The strong association of ID with all-cause mortality together with the high prevalence of ID in RTR identifies ID, even in the absence of anemia, as a possible target for treatment in these patients.

Anemia is common in RTR [15]. In the present study, in keeping with earlier reports, one-third of the population was anemic [4,16] and anemia was associated with mortality [14]. However, when adjusted for ID, the magnitude of the association of anemia with mortality decreased and lost statistical significance, possibly implicating that ID is one of the main driving forces of the association of anemia with mortality. Furthermore, after further adjustment for kidney function parameters, the association of anemia with mortality disappeared. In contrast, ID remained strongly associated with mortality, independent of both anemia and kidney function.

Traditionally, ID is clinically linked mainly to anemia. However, in addition to hemoglobin, iron is a key component of a number of cellular enzymes, for example, oxidases, catalases, and cytochromes, and other proteins such as myoglobin. As a result, iron is not only essential for oxygen transport but also plays a pivotal role in, for instance, the synthesis of DNA, electron transport, and cellular proliferation and differentiation [17,18].

Estimation of iron status is complex, and the cutoffs for the clinical diagnosis of ID are arbitrarily defined [19]. For ID, we used the definition commonly used (e.g. in CKD), TSAT < 20% and ferritin < 300 µg/l [20]. This definition includes both functional and absolute iron deficiency states and uses a combination of two frequently used iron markers, TSAT and ferritin [20–22]. As a sensitivity analysis for the definition of ID, we used another frequently used definition [13], basically rendering identical results. Also the individual iron status components (serum ferritin, TSAT, and serum iron) were associated with mortality, although the relationship between ferritin and mortality was nonlinear. When divided into quintiles, both high and low ferritin concentrations were associated with mortality. These findings are in concordance with studies in RTR and other patient groups [23,24]. These results point out to the possibility that both ID and iron overload may have deleterious effects on patient survival. It should be mentioned that serum ferritin concentrations are, in addition to iron overload, also elevated in other conditions such as inflammation, cardiovascular disease, and malignancies [23,25]. However, after adjustment for hs-CRP the association of high serum ferritin with mortality remained more or less unchanged. Indeed, iron overload is considered potentially harmful in nondialysis and dialysis patients receiving IV iron [26]. Recently, the pros and cons of intravenous iron therapy were evaluated during the KDIGO ‘Controversies Conference on Iron Management in Chronic Kidney Disease’ [19].

We did not investigate the mechanism(s) through which ID leads to increased mortality risk, and therefore, the causative mechanism can only be matter of speculation. Clinically, ID is associated with reduced cardiac performance and pulmonary hypertension. Indeed, post-transplant anemia is associated with worse outcomes such as more fatigue, reduced exercise capacity, lower quality of life, and higher incidence of congestive heart failure [27]. In agreement with this speculation adjusting for NT-proBNP (Table 3) diminished the association between ID and mortality, possibly

indicating that cardiac performance was one of the mediators between iron deficiency and all-cause mortality.

The strengths of our study is the cohort that was utilized for the analysis, a large cohort of well-characterized, stable RTR including extensive data on anthropometric and dietary factors, lifestyle, and medication use that allowed for adjustments for many confounders and without loss to follow-up.

One of the main limitations of our study is that a readily available clinical gold standard of functional and absolute ID does not exist. In some studies, in addition to markers of iron load (ferritin) and iron transport availability (TSAT), functional markers of iron incorporation in the red cell such as hypochromic red blood cells or reticulocyte hemoglobin content are used. However, these markers are difficult to use in clinical studies and are not routinely measured and superiority is not established over commonly used clinical definition. Another limitation is that we used a single baseline measurement of hemoglobin levels and iron status. However, most epidemiological studies use a single baseline measurement for studying the association of variables with outcomes, which as a consequence adversely affects the strength of the association of these variables with outcomes. In other studies, it was shown that when intra-individual variability of these variables was taken into account, a strengthening of the association with outcome, that is, coronary heart disease occurred as compared to the association of a single measurement [28,29]. As a consequence, it is likely that when including multiple measurements, the association of iron deficiency with mortality would be even more pronounced. Finally, as with any observational study, there may be unmeasured or residual confounding despite the substantial number of potentially confounding factors for which we adjusted.

In conclusion, we are the first to show that ID, independently of anemia, is associated with a higher risk of all-cause mortality in RTR. Further research is needed to reveal the mechanisms through which ID leads to higher risk of all-cause mortality. Moreover, further investigation is needed to assess whether analogue to heart failure patients, achievement of an adequate iron status, irrespective of hemoglobin levels, can be a possible new therapeutic target in RTR. As ID is a relatively easily modifiable factor, randomized controlled trials should focus on correction of ID in RTR in an effort to improve patient survival after transplantation.



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## Conflicts of interest

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